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First records of *Rhizopogon rocabrunae* and *R. pumilionum* (*Boletales*) from Italy

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Abstract — The paper reports a macro- and micromorphological investigation on *Rhizopogon rocabrunae*, a very rare hypogeous macrofungus, recently collected in Liguria (Italy) in two different times and locations. These specimens represent the first authentic record of this species from Italy. According to our microscopic analyses, an older Italian collection, formerly identified as *R. rocabrunae,* must be ascribed to *R. pumilionum*, a species previously never reported from Italy. Notes on closely related species are also provided.

Keywords — *Abies alba*, *Agaricomycetes*, *Rhizopogonaceae*, *Rhizopogon pannosus*, *Suillineae*

Introduction

The genus *Rhizopogon* Fr. encompasses hypogeous ectomycorrhizal fungi primarily associated with members of the *Pinaceae* Lindl. Phylogenetically monophyletic, the genus belongs to the so-called Suilloid radiation or suborder *Suillineae* of the *Boletales* (Grubisha et al. 2001, Binder & Hibbett 2006, Desjardin et al. 2008).

Until now, five species have been reported in Liguria (Northwest Italy), viz. *Rhizopogon luteolus* Fr. 1817, *R. occidentalis* Zeller & C.W. Dodge 1918, *R. rocabrunae*, *R. roseolus* (Corda) Th. Fr. 1909, and *R. villosulus* Zeller 1941. Among the above-mentioned species, *R. rocabrunae* is the least frequent in Liguria, previously reported in Italy on the basis of a single dubious collection (Montecchi & Sarasini 2000) and with few reports from Europe (Martín 1996, Cavet & Lopez 2004). Only two sites are known from Liguria, both located in Ligurian Maritime Alps (Alpi Marittime). One specimen was collected in

Testa d'Alpe forest, in an area dug by wild boars. Testa d'Alpe forest, which extends for 140 hectares from 750 to 1460 m. a.s.l., is the only forest in which the silver fir is considered native in Liguria. The other specimen was observed in an allochthonous silver fir forest, derived from a reforestation.

The paper evaluates the presence of *R. rocabrunae* in Italy based on the study of both the recent Ligurian collections and an older Italian collection (from Lombardia) formerly ascribed to *R. rocabrunae* (Montecchi & Sarasini 2000). Analysis of the old collection was motivated by the fact that Montecchi & Sarasini (2000) illustrated and described specimens with aberrant features for the species and did not mention some microscopic characters that are needed for a correct identification.

Materials and methods

Macroscopic and microscopic characters were described using a stereo microscope (Leica M 205 C) and a compound microscope (Axioscope, Zeiss), respectively. The description of the features is based on fresh and dry specimens (in the latter case after rehydration in water and lactic acid).

Microscopical observations were made from tissues mounted in distilled water, lactic acid plus acid fuchsine, 5% potassium hydroxide, and Melzer's reagent. For basidiospores and other structures at least 30 individuals were measured. The spore sizes are reported using three numbers corresponding to the minimum, average, and maximum values, respectively. The Qm abbreviation designates the average length to width ratio of the spores in side view.

Colour notations reported in brackets were taken from Kornerup & Wanscher (1978), indicated as "M." in front of a colour code. Identification references were Smith & Zeller (1966), Martín (1996), and Montecchi & Sarasini (2000).

All the Ligurian examined material is deposited and kept at GDOR (Herbarium of the Museo Civico di Storia Naturale Giacomo Doria, Mycologia section, Genova, Italy). Herbarium abbreviations follow Thiers (2010).

As concerns the geo-reference, a Garmin (eTrex Summit) Global Position System (GPS) was set to express the locations in WGS-84 coordinates in decimal degrees. The geographical data were mapped on the Official Map of Italian State (I.G.M.I) using GIS software (MapInfo 7.0). The data were also inserted in a database where all Ligurian macrofungi species are recorded.

Taxonomy

Rhizopogon rocabrunae M.P. Martín, Edic. Espec. Soc. Catalana Micol. 5: 95 (1996)

Description of the two collections from Liguria — Fig. 1

Basidiomata globose to subglobose, on average 3 cm in diam. Peridium well developed, 0.3–0.6 mm thick, brown with reddish to orange tinges (M. 6 B 8 C 5),

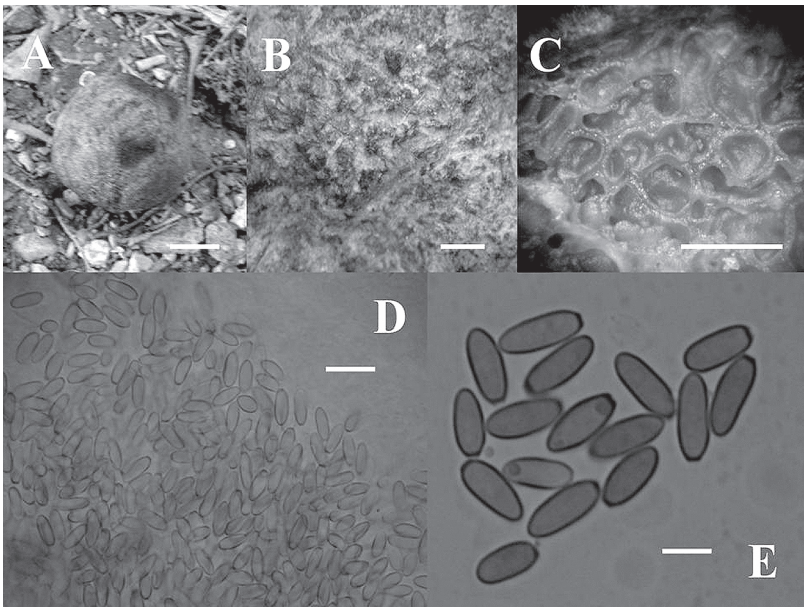

Figure 1. ***Rhizopogon rocabrunae***.
A. Ripe basidioma. B. Peridium surface. C. Gleba cells. D. E. Basidiospores.
Scale bars: A = 1 cm; B, C = 1 mm; D = 10 µm; E = 5 µm.

with evident, 0.15–0.35 mm thick squamules, at first orange (M. 6 B 8) then brownish to black. Rhizomorphs scarce, gray (M. 1 B, C 1) emanating singly from the base. Gleba firmly spongy with roundish to elliptical cells, 0.2 to 0.6 mm in diam., yellowish to brownish (M. 5 D 7). Tramal plates often in part gelatinized 100–200 µm thick. Smell and taste indistinct.

Peridium made up of hyaline, septate and thin-walled hyphae, 3–6 µm wide, encrusted with brown-orange pigment, with trend mostly parallel to the outer surface; squamules consisting of a more or less parallel arrangement of hyphae, with extracellular orange pigment. Basidia cylindrical, 4–10 spored. Basidiospores (6.6–)7.5(–9) × (2.3–)2.9(–3.5) µm, Qm = 2.586, elongated, ellipsoidal, smooth, often clearly truncated, transparent yellow to light green (1 A 4, B 4) when ripe, usually pluriguttulate. Clamp connections absent.

Habitat – solitary or gregarious under the needle layer of *Abies alba* Mill. Vernal.

Material examined: ITALY, Liguria, Foresta Demaniale di Gouta, Testa d'Alpe (IM), 1360 m a.s.l., G.P.S. (wgs 84) long 7.570027° lat 43.945343°, 17/06/2008, leg. M. Zotti, (GDOR 08061701); ITALY, Liguria, Bosco nero, Mendatica (IM) 1350 m. a.s.l., G.P.S. (wgs 84) long 7.733891° lat 44.125178°, 12/06/2008, numerous specimens, leg. G. Baiano (GDOR 08061201).

Description of the Sarasini collection from Lombardia
(cited in Montecchi & Sarasini 2000).

Basidiomata globose to subglobose, on average 1–2,5 cm in diam. Peridium color mostly brown (M. 7 F 6 – M. 6 C 4) with reddish tinges (M. 8 C 6). Gleba with olivaceous tinges (M. 8 C 2). Peridium made up of hyaline hyphae, with trend mostly parallel in the inner layer, while, in the outer layer at the squamules, consisting unordered parallel arrangement of hyphae. Basidiospores (6.5–)7.93(–9) × (2.2–)2.76(–3) µm, Qm = 2.87, elongated, ellipsoidal, smooth, not always clearly truncated, transparent yellow to light green (1 A 4, B 4).

Habitat – solitary or gregarious under the needle layer of *Pinus montana* Mill. In summer.

Material examined: ITALY, Lombardia, Valdidentro - Cancano (So), 10/08/1987, consisting of four specimens, leg. Aiana, det. Sarasini (AMB 267)

Discussion

Martín (1996) originally described *R. rocabrunae* from Spain based on two collections found under *Abies alba*. In the last ten years it has been reported from Italy under *Pinus montana* s.l. (Montecchi & Sarasini 2000) and from France in a *Picea alba-Abies alba* wood (Cavet & Lopez 2004).

The two Ligurian collections show features fitting very well the original description of *R. rocabrunae* (Martín 1996). This species is macroscopically characterized by a reddish orange squamulose peridium and microscopically by elongate basidiospores, clearly truncate at the base, and peridial squamules made up of hyphae running parallel to the peridium surface. The squamulose peridium gives the basidiomes a quite distinct and characteristically *Elaphomyces*-like appearance or, as reported by Martín (1996), resembling *Arbutus* berries.

Rhizopogon rocabrunae comes very close to *R. pannosus* Zeller & C.W. Dodge 1918, a North American species (Smith & Zeller 1966) reported also from Spain (Martín 1996) and recently from Switzerland (Kathriner & Mühlebach 2008). The latter species differs in having a more verrucose peridium with more irregular squamules made up of interwoven hyphae running perpendicular to the peridium surface, less gelified hyphae of the tramal plates, wider basidiospores (on average 3.4 µm, Qm = 2.3), different isoenzymatic and PCR-RFLP patterns (Martín 1996, Martín & Sánchez 1996, Moser & Peintner 2000), and an association with *Pinus* spp.

According to Moser & Peintner (2000), *R. pumilionum* (Ade) Bataille 1923 from the Austrian *Pinus montana* forests shares with *R. pannosus* the same structure of the peridial squamules, but it is distinguished by its narrower spores (on average 2.9 µm, Qm = 2.6; Moser et al. 1999, Moser & Peintner 2000). Preliminary studies based on 28S rDNA analysis by Jarosch (2001)

indicate that *R. rocabrunae*, *R. pannosus,* and *R. pumilionum* are closely allied but independent species.

The Sarasini collection from Lombardia labelled *R. rocabrunae* (Montecchi & Sarasini 2000) seems quite anomalous due to the ochraceous-coloured peridium and its association with *Pinus montana.* Microscopical analysis revealed that it is referable to *R. pumilionum* based on peridial squamules made up of tufts of ascending hyphae and on spore size. Additionally, the olivaceous tinged gleba and association with *P. montana* are features typical for this species (Moser & Peintner 2000). Therefore, this collection represents the first record of *R. pumilionum* from Italy.

The two Ligurian specimens represent the first authentic report of *R. rocabrunae* from Italy. Adding to the original Spanish and French collections, our records confirm that *Abies alba* seems to be the preferred ectomycorrhizal partner of *R. rocabrunae*, highlighting, as already pointed out in other *Rhizopogon* species as well as in related genera (e.g. *Suillus*), a rather strict, specific association between the mycobiont and the photosynthetic host in the *Suillineae* (Grubisha et al. 2001, 2002).

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